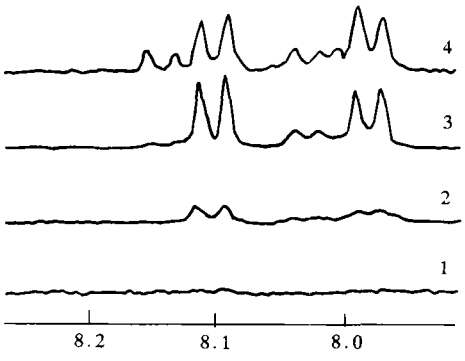

Fig. 4. ^{1}H-NMR spectrum of photosensitized oxidation of ClAlPcS to peptide (7.9—8.3 ppm). Irradiation time (min): 1, 0; 2, 20; 3, 40; 4, 70.

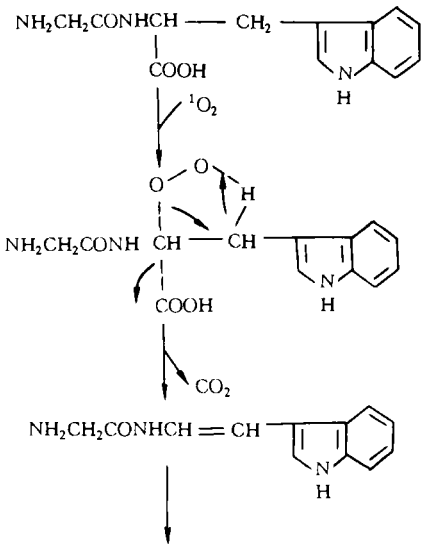

Fig. 5

destroyed when reaction is continued; (Ⅳ) the peptide is completely damaged.

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Genetic diversity of reared *N. miichthioides* population

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Abstract The genetic diversity of 30 reared *Nibea miichthioides* individuals was analyzed by random amplified polymorphic DNA (RAPD) with 20 random primers. The result showed that the genetic diversity of reared individuals was relatively low with 15.31% polymorphism and 0.031 9 of the average difference (AD). The result also indicated that RAPD is a useful way in genetic diversity analysis of fish population.

Keywords: ***Nibea miichthioides*, genetic diversity, RAPD.**

N. MIICHTHIOIDES has recently become a new kind of rearing species in southern China for its high

growth rate, disease-resistance and tastiness. The fry number of wild *N. miichthioides* decreased by 80% in 1993 as great quantities of those wild fry had been captured for box-net rearing since 1990, and few wild fry could be caught later. To meet the requirement of the intensive farming, larvae breeding is widely prevalent in southern Fujian and Guangdong provinces. As small numbers of wild adults were used in artificial breeding, it has caused the genetic "bottle-neck" which resulted in the loss of high quality properties and genetic resourse degeneration. In order to protect and develope the fine traits of the rearing groups and avoid the genetic depression, it is necessary to make a study on the genetic diversity of the existing population.

Random amplified polymorphic DNA (RAPD), based on the polymerase chain reaction (PCR) and developed in the early 1990s, is a new technique for genetic study. It is widely used in systematic zoology[1—3], gene location[4], resource assession[5—7], and so on. However, it has just been applied to the fishery systematic classification and breeding since 1995 in China, no paper has been published on fish genetic diversity. In this note, we studied the genetic diversity of sea fish by RAPD.

1 Materials and methods

(ⅰ) Materials. Thirty reared F_1 individuals bred from wild *N. miichthioides* adults in June 1996 were collected from the box-net located in Huoshao Island, Xiamen on July 25, 1997. The mean length was 45.3 cm (41.1—49.0 cm) and the mean weight was 901.5 g (600—1 200 g). Primers containing 20 species (table 1) from Operon Company were used for RAPD.

Table 1 Sequences and operon codes of 20 random primers used for RAPD

Primer	Sequences (5→3)	Primer	Sequences (5→3)
OPV-01	TCACCGATGG	OPV-11	CTCGACAGAG
OPV-02	AGTCACTCCC	OPV-12	ACCCCCCACT
OPV-03	CTCCCTGCAA	OPV-13	ACCCCCTGAA
OPV-04	CCCCTCACGA	OPV-14	AGATCCCGCC
OPV-05	TCCGAGAGGG	OPV-15	CAGTGCCGGT
OPV-06	ACGCCCAGGT	OPV-16	ACACCCCACA
OPV-07	GAAGCCAGCC	OPV-17	ACCGGCTTGT
OPV-08	GGACGGCGTT	OPV-18	TGGTGGCGTT
OPV-09	TGTACCCGTC	OPV-19	GGGTGTGCAG
OPV-10	GGACCTGCTG	OPV-20	CAGCATGGTC

(ⅱ) Methods. The ways to isolate fish DNA and for electrophoresis in present study were applied by the method described in ref. [8]. The agarose gel concentration was 1.5% and the buffer was Tbe 0.5X (pH 8.0). Fish DNA amplifications were carried out by using Gene Amp. system 9600 (PE Co.). The reaction mixture (25 μL) consisted of 1.5 μL primer (table 1), 1 U Taq, 1 μL dNTP (2.5 mmol), 1.8 μL Mg^{2+} (25 mmol), 2.5 μL 10× buffer, 16 μL H_2O, 2 μL template DNA. The amplification precedure was as follows: 94℃ for 2 min → (93℃ for 1 min →36℃ for 1 min→72℃ for 2 min)×45 recycles →72℃ for 5 min. RAPDistance Package-Version 1.04 software was used to deal with the data. Similarity between individuls was analyzed as: $S = 2N_{xy}(N_x + N_y)$ followed Nei (1979), where S = similarity. In order to check up the reproducibility of RAPD, a series of amplication reactions were carried out by using various template DNAs, different concentrations, purifications and different tissues from experimental *N. miichthioides*. Also, the affected factors on RAPD test were discussed in the present paper.

2 Results and discussion

(ⅰ) Effects of DNA samples isolated from various tissues, different purification treatments and concentrations on RAPD.

(1) Purifications of DNA templates. Table 2 and fig. 1 show the DNA samples purified in different ways and their consequent results.

Table 2 Amplification efficiency of DNA with different treatments

No.	RNase treatment	Ethanol precipitate	Amplification efficiency	$A_{260/280}$
1	N[a)]	twice	clear	1.741
2	Y	twice	clear	1.823
3	Y	none	diffusive	1.239
4	Y	once	clear	1.764
5	Y	twice	clear	1.823

a) N, untreated by RNase; Y, treated by RNase.

The amplification products of samples 1 and 2 had a similar pattern (both had 4 bands), but the bands of sample 1 are more clear than that of sample 2. There were only 3 bands in sample 3 with serious diffusive background. However, both samples 4 and 5 had a similar product pattern generated by RAPD. All the above indicated that RNA has influence on the DNA content (i.e. on the ratio of $A_{260/280}$) but not on DNA amplification efficiency; DNA samples precipitated by ethonal for only once is pure enough for their RAPD reaction, which is little disturbed by pureness.

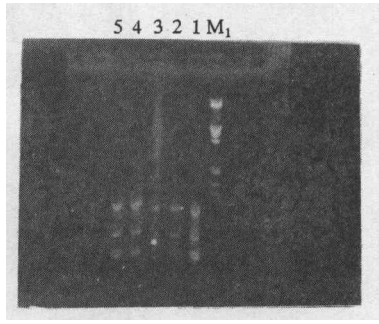

Fig. 1. RAPD products of *Nibea miichthioides* template with different purity.

(2) Template DNA concentration. Effects of various DNA contents on RAPD patterns are evidently shown in fig. 2. 10 different contents ranging from 0.01 to 1 mg/μL of *N. miichthioides* genetic DNA were arranged to run on a 1.5% agarose gel. The differences of the patterns generated by RAPD were little by using the template concentrations ranging from 0.05 to 50 ng/μL. It is evident that DNA RAPD reaction is so sensitive that the trace sample (0.05 ng/μL) could be detected clearly, and it would be greatly disturbed or even failed if the template concentration is more than 100 ng/μL.

(3) Comparison of the DNA templates isolated from different tissues by RAPD reaction. All the 4 DNA templates isolated from fish muscle, blood, spermary and fin displayed similar RAPD fragment patterns by RAPD reaction (fig. 3). It means that template DNA samples could be isolated from the fin or other unfatal organs while the fish were kept alive still; that is, it possesses practical significance that a large amount of DNA samples essential for the population genetics analysis could be obtained by not destroying living animals.

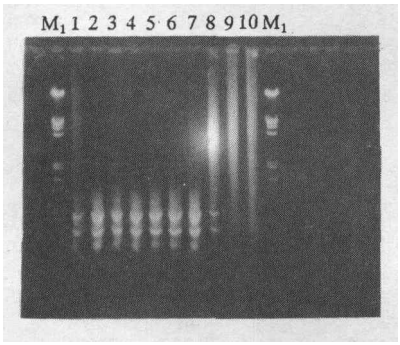

Fig. 2. RAPD products of *N. Miichthioides* template with different concentrations.

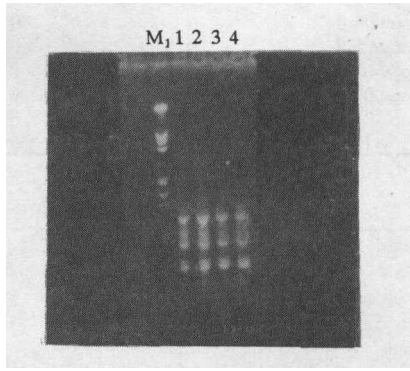

Fig. 3. RAPD products of *N. Miichthioides* template extracted from different issues. M_1, λDNA EcoR Ⅰ/Hind Ⅲ marker.

(ⅱ) Population genetics analysis. Each of the 20 primers, except opv-10, 11 and 14, generated 2—10 bands by RAPD (fig. 4). The effective 17 primers produced the total 111 bands, with an average of 6.5 bands per primer. The molecular weight of the amplified bands ranged from 500 to 2 500 bp. Of the 111 bands, only 17 displayed the polymorphic loci, the ratio of which is 15.31%. All the polymorphic loci and their relative frequencies are shown in table 3, and the average similarity (AS) of the individuals is given in table 4. The AS of the reared group was 0.968 and its average difference (AD) was 0.031 9. All the data above demonstrated that the level of polymorphism and genetic variability of this

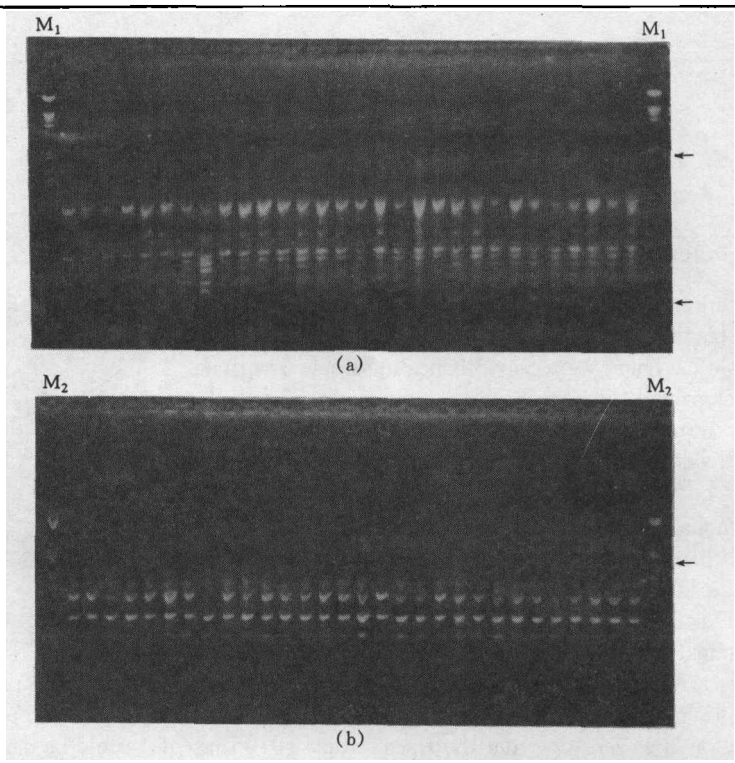

Fig. 4. The electrophoresis patterns of RAPD products of 30 *N. Miichthioides* genomertic DNA with primers OPV-15 and OPV-20. The arrow shows the polymorphic locus. M_2, PGEM DNA marker.

Table 3 Characteristics of amplification products of polymorphic loci and their relative frequencies

Position of polymorphic loci	Length of fragment/kb	f [a)]	Position of polymorphic loci	Length of fragment/kb	f
opv-02-4	0.80	0.767	opv-13-1	1.02	0.467
opv-02-8	0.60	0.867	opv-13-2	0.85	0.833
opv-04-1	1.60	0.767	opv-14-1	1.90	0.733
opv-04-3	0.90	0.767	opv-15-1	1.50	0.833
opv-05-8	0.52	0.533	opv-16-1	2.00	0.800
opv-07-3	1.00	0.633	opv-18-7	0.56	0.700
opv-07-6	0.75	0.633	opv-20-1	1.95	0.667
opv-08-6	0.65	0.700	opv-20-10	0.67	0.233
opv-12-2	1.20	0.233			

a) f, amplified frequency of polymorphic loci = samples with polymorphic loci to total samples.

Table 4 AS_i [a)] between the individuals of reared *N. miichthioides* population

Sample	1	2	3	4	5	6	7	8	9	10
AS_i	0.966	0.974	0.958	0.967	0.968	0.971	0.964	0.956	0.977	0.974
Sample	11	12	13	14	15	16	17	18	19	20
AS_i	0.967	0.974	0.971	0.973	0.966	0.965	0.968	0.961	0.968	0.967
Sample	21	22	23	24	25	26	27	28	29	30
AS_i	0.972	0.974	0.955	0.970	0.961	0.962	0.971	0.973	0.971	0.973

a) AS_i, mean value of a single sample to the others, $AS_i = 1/(n-1) \times \sum_{i=1}^{n} S_i$, n means the total number of samples; $AS = 1/n \sum_{i=1}^{n} AS_i$.

reared population was low, its average polymorphic value 15.31% was much lower than that of average value 24.7% in vertebrate, ranging from 14.5% to 33.6%. It has been verified in many animals that the level of the genetic variability of reared group is usually lower than that of the wild population, because of some affecting factors such as genetic bottle-neck, genetic drift and inbreeding depression which were inevitable in the reared process[5,9—11]. The 30 *N. miichthioides* in this study were collected from the same batch progeny bred in 1996, and their parents used in this breeding process were usually less than 20

adults. So it might be the main reason that the lower diversification of the reared *N. miichthioides* would result in genetic bottle-neck. However, it is worth pointing out that in these 30 individuals, none of a pair of bands was completely identical, the range of their genetic difference was from 0.004 61 to 0.063 41, showing that this reared population still possesses a widely potential genetic variability. Suggestions for keeping sustainable utilization of *N. miichthioides* resource were proposed as follows:

(i) Great importance should be attached to maintenance of fine natural ecosystems and protection of genetic variability of wild population.

(ii) Genetic variability of rearing population by effective management must be preserved, and the negative influence of genetic bottle-neck, genetic drift and resource depression must be avoided.

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Three different viruses observed from the tissues of diseased mandarin fish *Siniperca chuatsi*

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Abstract Three different kinds of viruses, the spherical virus SCSV with a diameter of about 280 nm, the rhabdovirus SCRV with a size about 250 × 120 nm, and the baculovirus SCBV with a size about 200 × 100 nm, were observed from the tissues of diseased mandarin fish *Siniperca chuatsi* with outbreak of infection and acute lethality. This phenomenon implicated that the reason why the epizootic disease of mandarin fish could not be quenched by only one kind of virus vaccine can be explained by the fact that the fish may be infected by different kinds of viruses. Therefore, more attention should be paid to the complexity of virus pathogens in the prevention strategy for mandarin fish diseases.

Keywords: mandarin fish, virus, viral disease, pathogen.

MANDARIN fish *Siniperca chuatsi* is a special aquaculture fish in China. Unfortunately, the mortality was